16.

Serologic Relationships among Bovidae and Cervidae.

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INTRODUCTION.

A difficulty which confronts the serologist in studies of animal relationships is lack of blood specimens of the uncommon animals. Most mammalian serological studies have been made on the bloods of the smaller animals and common domesticated forms. The author has been fortunate in securing the sera of some uncommon animals among which were several species of the order Artiodactyla. These bloods were obtained through the efforts and kindness of Dr. C. R. Schroeder of the New York Zoological Park. The present report emphasizes the relationships among eleven species belonging to the families Bovidae and Cervidae and includes data of cross reactions with other Artiodactyla and two Perissodactyla.

Ehrhardt (1929) gave a complete review of the research done to 1927 on the serological relationships of animals by means of the serum precipitin test. The first report on Artiodactyla blood was that of Myers (1900) who described reactions of antisera produced by injections of the globulin fraction of ox and sheep bloods. Uhlenhuth (1901) injected rabbits intraperitoneally with defibrinated ox blood and reacted the antiserum with the blood of fifteen different species of mammals. Nuttall & Dinkelspiel (1901) noted that anti-ox serum gave weaker reactions with the blood of sheep than with ox. Nuttall (1904) in his monograph "Blood Immunity and Blood Relationships," described many qualitative and quantitative tests, the latter being done in cooperation with Strangeway. These tests included bloods from many species of Bovidae and Cervidae. A number of other workers since Nuttall's report have used the bloods of the more common domesticated Bovidae but very little data has been accumulated with the blood of Cervidae. A general conclusion that could be arrived at from all the work done is that the Bovidae and Cervidae show a close serological affinity to each other though the animals of these two families often could be distinguished from each other. Moreover, at times, within the family Bovidae, the ox could be distinguished from the sheep or goat. The results have not been sufficiently consistent and these discrepancies have prevented the serological method from being considered as useful as morphological or other criteria in taxonomic and phylogenetic studies.

Factors that modify the serum precipitin reaction in regard to relationship studies and methods which yield more consistent results were not formulated until recent years. Boyden (1926) stressed the importance of controlling physical and chemical factors in the *in vitro* tests. He devised the principle of "reciprocal relationships." Wolfe (1933) further elaborated on this and showed that greater specificity (1935, 1936) could be secured by regulating the amount of antigen injected into the antibody producer, namely, the rabbit. Wolfe (1939) found that injecting minimum quantities of antigen necessary for antibody production would yield, with a fair consistency,

antisera that could be used in distinguishing very closely related forms. It was emphasized in this report that there was a necessity for uniformity and standardization of the known factors by serological workers. It was also shown that discrepancies of relationship values within his own data and probably the data of other workers was often not due to the technique but due to the variance in antibody productivity of the rabbit. Thus it was necessary to secure as many antisera as possible to insure reliable results.

Methods and Materials.

Nineteen healthy adult rabbits were injected with the blood serum of eight different species of animals of the order Artiodactyla. Fifteen of the animals were given a series of 3 injections with a standardized antigen solution containing 2 mg. protein per cubic centimeter. The injections were made on alternate days and were given in increasing dosages. The total quantity injected varied from 0.75 to 1.5 mg. per kilo body weight. If this did not result in antisera of sufficient potency the rabbits were given 2 additional injections with a quantity equal to one-half the amount given in the first series. It was necessary to give one of the rabbits a third series of injections in order to secure a high titered antiserum. Several rabbits failed to produce

Table 1.
Antiserum production data.

Rabbit No.	*Antigen injected*	*Series of injections*	*Amount injected mg. per kilo body weight*	*Bled — days after last injection*	*Titer*
A2	Sheep	1	1. cc. undiluted		not used
		1*	.5	8	512,000
71		1	1.5	10	not good
			.75	9	512,000
41	Tahr	1	1.5	10	512,000
42		1	1.5	10	256,000
50	Goat	1	25.	9	512,000
51		1	25.	9	256,000
45	Black buck	1	.75	10	512,000
49	antelope	1	.75	14	256,000
72		1	1.5	11	256,000
73		1	1.5	11	256,000
55	Eland	1	1.5	10	512,000
64		1	1.5	9	384,000
A12	Axis deer	1	1.5	9	512,000
61		1	1.5	9	512,000
35		1	1.	10	not good
		1	.5	10	not good
		1	.5	9	1,024,000
46	Elk	1	1.5	10	1,024,000
48		1	1.5	10	1,024,000
57		1	1.5	10	not good
		1	.75	10	256,000
62	Virginia deer	1	25.	10	512,000

* 2 injections given 10 months after 1st series.

antisera of the desired titer (1:128,000 after 1 hour of incubation). Four rabbits were injected with larger quantities of antigen. These animals (except A2) were to be reinjected for another type of experiment but the quantity injected in the first series was small enough to give a fairly specific antiserum (Wolfe, 1935). Table 1 records the antiserum production data.

The test antigens used for relationship studies were the blood sera of 15 species of Artiodactyla and Perissodactyla and these are listed in Table 2. Since the amount of antisera and antigens on hand was not always sufficient, each antiserum was not tested with each antigen. Moreover, some of the antigens were received after the *in vitro* tests were made. One-half cubic centimeter of a standard antigen solution containing 2 mg. (1:500 dilution) of protein (based on total nitrogen) was serially diluted in serological test tubes. Layered beneath the antigen was 0.1 cc. of the antiserum which was used either undiluted or diluted. The degree of dilution for the different antisera was not constant but the dilution listed in the relationship tables was that one which gave the greatest differentiation of the heterologous reactions without affecting the clearness of the end point. At times slightly higher dilutions than those recorded so altered both the homologous and heterologous titers that the readings and results were not trustworthy.

The tests were made in duplicate and were incubated in a water bath at a temperature of 37.5° C. ± 1° C. Readings were taken at 1, 5, 10, 20, 30 and 60 minutes. The titer of the reaction was the highest dilution of the antigen at each interval of time that gave a "ring" at the region of contact of antigen and antiserum. In relationship studies the cross-reactions were calculated in per cent. of the homologous titer. When the homologous titers were 1:64,000 or less the results are unsatisfactory for then low heterologous titers resulted in high percentage values. The error in reading the end point was plus or minus one tube or a possible error of 100%, which is not great for such a sensitive reaction.

The degree of relationship among the animals could be determined at times by the 60 minute readings with undiluted sera. It was often necessary to use either the time factor (readings at intervals up to an hour) or various

TABLE 2.
Source of test antigens.

Family	*Scientific name*	*Common name*	*Source of material*
Bovidae	*Bos taurus*	Ox	Local Packing Co.
	Bison bison	American buffalo	Yellowstone Park
	Taurotragus oryx	Eland	N. Y. Zoo.
	Antilope cervicapra	Black buck antelope	N. Y. Zoo.
	Hippotragus niger	Sable antelope	N. Y. Zoo.
	Ovis aries	Domestic sheep	Local Packing Co.
	Capra hircus	Domestic goat	University Farm
	Hemitragus jemlahicus	Tahr	N. Y. Zoo.
Cervidae	*Axis axis*	Axis deer	N. Y. Zoo.
	Cervus canadensis	American elk	N. Y. Zoo.
	Odocoileus virginianus	Virginia deer	Madison Zoo.
Suidae	*Sus serofa*	Domestic pig	Local Packing Co.
Hippopotamidae	*Choeropsis liberiensis*	Pigmy hippopotamus	N. Y. Zoo.
Camelidae	*Llama huanacos*	Llama	Madison Zoo.
Equidae	*Equus caballus*	Horse	University Farm
	Equus caballus (*mulus*)	Mule	Fur Farm

dilutions of antiserum or both factors in order to differentiate the bloods. Control of these factors, it is known, increases the specificity of the reaction (Nuttall, 1904, p. 142; Wolfe, 1939).

Experimental Data.

Table 3 records the reactions of 2 anti-tahr sera. Serum No. 41 was less specific than No. 42. Two series of tests were made with the former. In one

Table 3.
Reactions (titers*) of 2 anti-tahr sera.

Antigen	*Serum No. 41*									
	5 min.		*10 min.*		*20 min.*		*30 min.*		*60 min.*	
	Antiserum dilution									
	1:1	*1:3*	*1:1*	*1:3*	*1:1*	*1:3*	*1:1*	*1:3*	*1:1*	*1:3*
Tahr	32	12	54	64	128	128	256	128	512	256
Goat	8	2	32	2	128	64	256	64	256	128
Sheep	16	1	64	3	128	64	256	128	256	128
Sable Antelope	4	4	16	16	64	16	128	16	128	48
Black Buck Antelope	2	0	6	2	16	8	128	16	256	16
Ox	4	1	8	4	8	8	96	16	256	16
Buffalo	3	1	6	4	6	8	64	8	256	8
Eland	2	0	4	0	6	2	64	4	128	4
Virginia Deer	1	0	1.5	0	1.5	0	1.5	0	3	0
Axis Deer	1.5		3		4		6		6	
Elk	3	0	8	0	8	0	12	0	12	2
Pigmy Hippopotamus	0		0		0		0		0	
Pig	0		0		0		0		0	
Horse	0		0		0		0		0	

Serum No. 42
Antiserum dilution = 1:0.5

Tahr	8	32	128	128	256
Goat	0	0	4	16	64
Sheep	0	0	4	16	96
All other tests were negative					

* All titers are in thousands.

series the antiserum was diluted with an equal volume of saline (1:1 dilution) and in the other series it was diluted with three volumes of saline (1:3 dilution). The higher dilution resulted in much weaker reactions at the 5 and 10 minute readings for all the heterologous antigens. With this same dilution the titers at 20, 30 and 60 minutes remained weak for the heterologous tests, with the exception of the sheep, goat and sable antelope. Thus the titers of the tahr, sheep and goat antigens were relatively of the same order at the 5 or 10 minute reading when the antiserum was of a 1:1 dilution but

TABLE 4.

Relationship values (in per cent.) of 2 anti-tahr sera.

Antigen	*Serum No. 41*					
	10 min.		*30 min.*		*60 min.*	
	Antiserum dilution 1:1	1:3	*Antiserum dilution* 1:1	1:3	1:1	1:3
Tahr	100.	100.	100.	100.	100.	100.
Sheep	50.	3.12	100.	50.	50.	50.
Goat	100.	4.68	100.	100.	50.	50.
Sable Antelope	25.	6.25	50.	12.5	25.	18.75
Black Buck Antelope	9.37	3.12	50.	12.5	50.	6.25
Ox	12.5	6.25	37.5	12.5	50.	6.25
Buffalo	9.37	6.25	25.	6.25	50.	3.12
Eland	6.25	0	25.	3.12	25.	1.56
Elk	12.5	0	2.34	0	2.34	.39
Axis Deer	4.67	——	3.12	——	1.17	——
Virginia Deer	2.34	0	.58	0	.58	0
Pigmy Hippopotamus	0		0		0	
Pig	0		0		0	
Horse	0		0		0	
Llama	0		0		0	

Serum No. 42
Antiserum dilution = 1:0.5

Tahr	100.	100.	100.
Sheep	0	12.5	25.
Goat	0	12.5	37.5
All other tests were negative.			

with the higher dilution the latter two were easily distinguished from the tahr blood. The reactions of all the other Bovidae were high at the 30 and 60 minute intervals when the more concentrated antiserum was used, but upon a higher dilution the reactions of all, with the exception of the sable antelope, decreased considerably. The reactions with the Cervidae blood were generally much weaker than those of the Bovidae at both dilutions and at all time intervals.

Serum No. 42 was exceedingly specific. It gave reactions only with the sheep and goat, these reactions being weak except at the 60 minute reading.

Table 4 shows the degree of relationships of the 2 anti-tahr sera based on the data of Table 3. The values for 10, 30 and 60 minutes were picked arbitrarily for they illustrate best the effect of both the dilution and the time factors. Serum No. 41 shows that tahr, sheep and goat are very closely related. This follows their accepted classification for they are members of the same subfamily Caprinae. The data also indicate that the sable antelope is more closely related to the tahr than are the other members of the Bovidae

TABLE 5.

Relationship values (in per cent.) of 2 anti-sheep and 2 anti-goat sera.

Antigen	*Anti-sheep A2 diluted 1:4* *Anti-sheep 71 undiluted*			*Anti-goat 50 diluted 1:0.5* *Anti-goat 51 diluted 1:0.5*		
		30 min.	*60 min.*		*30 min.*	*60 min.*
Sheep	A2* 71*	100. (256) 100. (256)	100. (512) 100. (512)	50 51	100. 100.	100. 100.
Goat	A2 71	100. 12.5	50. 25.	50* 51*	100. (256) 100. (128)	100. (512) 100. (256)
Tahr	A2 71	100. 12.5	100. 25.	50 51	100. 100.	100. 100.
Sable Antelope	A2 71	50. 6.25	25. 25.	50 51	50. 100.	25. 50.
Black Buck Antelope	A2 71	25. 1.56	25. 1.56	50 51	12.5 12.5	12.5 12.5
Ox	A2 71	12.5 1.56	37.5 1.56	50 51	6.25 12.5	6.25 18.75
Buffalo	A2 71	18.75 1.56	50. 1.56	50 51	6.25 12.5	12.5 25.
Eland	A2 71	—— 3.12	—— 3.12	50 51	3.12 4.68	6.25 6.25
Elk	A2 71	—— .78	—— .78	50 51	3.12 3.12	6.25 6.25
Axis Deer	A2 71	6.25 1.56	12.5 .78	50 51	3.12 6.25	6.25 3.12
Virginia Deer	A2 71	6.25 1.19	25. .58	50 51	6.25 2.34	6.25 6.25

* The number in parenthesis is the titer in thousands. Heterologous titers may be calculated from the per cent. of relationship.

with the exception of the goat and sheep. The bloods of the Cervidae showed a definite but a more distant affinity to the tahr. The negative results with pig, pigmy hippopotamus and horse do not indicate a lack of relationship but emphasize that specific antisera can be produced that will give only "group" or "subgroup" reactions (Wolfe, 1935, 1936).

The relationship values with serum No. 42 were limited to the most closely related forms because of the great specificity of the serum. The closeness of the goat and sheep to the tahr, demonstrated by serum No. 41, was verified by the reactions of this second serum.

The reactions of two anti-sheep and two anti-goat sera are recorded to-

TABLE 6.

Relationship values (in per cent.) of 2 anti-eland sera.

Antigen	*No. 55 diluted 1:0.75; No. 64 diluted 1:3*						
		1 min.	*5 min.*	*10 min.*	*20 min.*	*30 min.*	*60 min.*
Eland	55*	0 (0)	100.(32)	100.(128)	100.(256)	100.(512)	100.(512)
	64*	100.(12)	100.(128)	100.(128)	100.(192)	100.(384)	100.(384)
Ox	55	0	3.12	3.12	1.56	12.5	25.
	64	0	25.	50.	75.	37.5	75.
Buffalo	55	0	3.12	1.56	1.56	12.5	50.
	64	0	50.	100.	100.	75.	75.
Black Buck Antelope	55	0	0	.78	.58	.78	2.34
	64	0	0	25.	75.	37.5	75.
Sable Antelope	55	0	0	0	1.56	1.56	3.12
	64	no tests	made				
Sheep	55	0	0	0	.39	.39	.78
	64	0	0	0	18.75	37.5	37.5
Goat	55	0	0	0	.39	.39	.39
	64	0	0	0	37.5	37.5	75.
Tahr	55	0	0	.78	.58	.39	.58
	64	0	0	0	18.75	18.75	37.5
Elk	55	0	0	0	.39	.39	.78
	64	0	0	25.	75.	75.	75.
Axis Deer	55	0	0	.78	.78	.39	.39
	64	0	0	25.	37.5	37.5	75.
Virginia Deer	55	0	0	.78	.78	.39	.78
	64	0	0	25.	37.5	37.5	37.5
Mule	55	all tests	negative				
	64	no tests	made				
Pigmy Hippopotamus	55	all tests	negative				
	64	no tests	made				
Pig	55	all tests	negative				
	64	no tests	made				

* The number in parenthesis is the titer in thousands. Heterologous titers may be calculated from the per cent. of relationship.

gether in Table 5. Serological tests have shown that the bloods of these two animals are very closely related and usually indistinguishable by the precipitin method. Wolfe (1933) emphasized that when the bloods of two or more animals are very closely related their heterologous reactions should be similar. The data further verify this principle. The antisera of the two different species gave reactions that indicated the very close affinity of the tahr to them and also showed that the sable antelope, though not as closely related to the goat and sheep as is the tahr, has a closer affinity to them than do the other Bovidae or the Cervidae. The anti-sheep sera brought out this fact more clearly than did the anti-goat sera; this may be due to the fact that the former antisera were produced by the injection of a smaller quantity of antigen, which often results in more specific antisera. The anti-sheep sera also gave definite Bovidae-group reactions but the less specific anti-goat sera did not.

Two anti-eland sera (55 and 64) of different specificity were produced by injections of minute quantities of antigen. The relationship values of these two antisera are recorded in Table 6 and they indicate that the ox and buffalo are more closely related to the eland than are some other members of the Bovidae. Serum 55 was the more specific antiserum and its reactions at the intervals of time at which the titers were read were greater with the ox and buffalo antigens than they were with other Bovidae or with the Cervidae. On the other hand, serum 64 showed a closer kinship of the ox and buffalo to the eland only at the 5, 10 and 20 minute titers while the 30 and 60 minute readings were not significantly different from those of other Bovidae or the Cervidae.

Serum 55 gave weak reactions but of similar magnitude with the three species of Cervidae and the Bovidae except the ox and buffalo. On the other

TABLE 7.

Relationship values (in per cent.) of anti-black buck antelope antisera*.

Antigen	*No. 45 Diluted 1:0.5*	*No. 49 Undiluted*	*No. 72 Undiluted*	*No. 73 Undiluted*
Black Buck Antelope	100.(516)†	100.(256)	100.(256)	100.(256)
Sable Antelope	6.25	0	12.5	6.25
Sheep	6.25	0	12.5	3.12
Goat	3.12	0	12.5	1.56
Tahr	3.12	0	6.25	1.56
Ox	3.12	0	3.12	3.12
Buffalo	.78	0	3.12	1.56
Eland	1.56	0	3.12	.78
Elk	.78	0	.78	.78
Axis Deer	.39	0	1.56	.78
Virginia Deer	.78	0	.39	.39

† The number in parenthesis is the titer in thousands. Heterologous titers may be calculated from the per cent. of relationship.

* All are 60 minute values.

hand, with serum 64 the Cervidae reactions were very definite at 10 minutes, but the sheep, goat, tahr and black buck antelope reactions were not noticed until the 20 minute period. This difference in reaction was evident when one volume of the antiserum was diluted with three volumes of buffered saline but not if it were used undiluted or diluted with one volume of saline. These results may indicate that the bloods of the Cervidae used are more closely related to the eland than certain members of the family to which the eland belongs according to its present classification. Since only one of 2 antisera gave these results it is, of course, necessary to secure additional data.

TABLE 8.

Relationship values (in per cent.) of 3 species of Cervidae.

Antiserum	*Antiserum No.*	*Antiserum dilution*	*Time of reading (min.)*	*Axis Deer*	*Elk*	*Virginia Deer*
Axis Deer	A12	1:1	5	100. (16)	100.	0
			10	100. (64)	75.	50.
			20	100. (192)	100.	75.
			30	100. (256)	100.	75.
			60	100. (512)	75.	75.
		1:4	20	100. (8)	50.	50.
			30	100. (128)	100.	3.12
			60	100. (128)	100.	100.
	35	1:2	5	100. (2)	25.	0
			10	100. (128)	50.	1.56
			20	100. (384)	75.	37.5
			30	100. (1024)	25.	12.5
			60	100. (1024)	25.	12.5
	61	1:3	5	100. (64)	100.	50.
			60	100. (512)	100.	25.
Elk	46	1:3	5	100.	100. (256)	25.
			60	50.	100. (1024)	25.
	48	1:3	5	50.	100. (128)	25.
			60	100.	100. (1024)	25.
		1:5	5	(1)	(2)	(0)
			60	50.	100. (128)	3.12
	57	1:0.5	5	37.5	100. (128)	3.12
			10	50.	100. (256)	25.
			60	100.	100. (256)	75.
		1:2	60	50.	100. (128)	12.5
Virginia Deer	62	1:1	5	50.	50.	100. (128)
			60	75.	75.	100. (512)
		1:3	10	0	0	100. (64)
			20	50.	50.	100. (128)
			60	50.	50.	100. (256)
		1:4	10	0	0	100. (16)
			20	0	0	100. (64)
			60	50.	50.	100. (256)

* The number in parenthesis is the titer in thousands.

Four antisera were produced by injections of minimum quantities of black buck antelope serum; these were all very specific. The cross reactions with Bovidae and Cervidae were much weaker than the homologous tests. Antiserum 49, though showing a high homologous titer, failed to give reactions with any of the heterologous antigens. Further results indicate that the sable antelope, sheep, goat, and tahr were more closely related to the black buck antelope than were the other Bovidae or Cervidae. Beddard (1920) states, "It is exceedingly difficult to separate antelopes from the sheep, oxen and goats. Their inclusion along with these creatures in one family, Bovidae, shows that no differences of an important character exist. . . . It is perhaps with the goats that the antelopes have their nearest affinities." The correlation of present serological findings with the statements of Beddard based on morphological data is highly significant.

The reactions of black buck antelope with the ox, buffalo and eland, though slightly greater, were not significantly higher than those with the Cervidae. It is possible that the less specific antisera might have demonstrated the relationships better than the antisera that were used. The high degree of specificity of the reactions has somewhat obscured the kinship involved and attempts should be made to further verify the present findings by the use of slightly less specific antisera.

The Cervidae antisera reactions furnished the interesting results shown in Table 8. The only reactions listed are the homologous ones and the cross reactions with the bloods of the other two deer, although tests were also made with eight species of Bovidae. The three axis deer and three elk antisera yielded reactions that usually failed to distinguish these two species. The greater number of relationship values for the cross tests with the elk or axis deer were 50 to 100 per cent., only a few being 25 and 37.5 per cent., which is just outside the limits of error of the tests. On the other hand, the titers of certain of the antisera of these two species with the Virginia deer serum were often so much lower than the homologous titers that the latter could easily be distinguished from either the axis or elk. This fact is especially well shown with the anti-axis deer serum A12 diluted 1:4, anti-axis deer serum 35 diluted 1:2, anti-elk serum 57 diluted 1:5 and anti-elk serum 48 diluted 1:2.

Only one anti-Virginia deer serum was produced. This serum when diluted with 3 volumes of saline could differentiate the Virginia deer serum from that of either the elk or axis deer at the 10 minute reading. A 1:4 dilution of the antiserum retarded the positive reaction with the two heterologous bloods to the 60 minute reading, while with the homologous antigen the reactions were positive though of low titer at 10 and 20 minutes.

These results, which show an exceedingly close similarity of the blood of elk to that of the axis deer and a distinction of both of these from the Virginia deer, are very significant, for they can be correlated with the origin of these animals. According to Scott (1937, p. 322), the American elk (also called wapiti) and the Virginia deer are North American forms that have had a different origin. Scott states that, ". . . North American deer form two strongly contrasted groups, the northern and southern. In the northern group the deer are like those of the Old World—these include the Wapiti." The southern group, which includes the Virginia deer, seems to have had a different ancestry and, according to Scott, probably originated from a "long line of American ancestry."

The per cent. values of the anti-deer reaction with the Bovidae were usually much lower than that with the homologous blood. In a small number of tests the titers were high enough so that a distinction was not possible, but most often the percentage of reaction was 12.5 per cent. or lower. The indications were that the ox and buffalo were more closely related to the deer group than were the other Bovidae.

Discussion.

The precipitin test, a serological method, was used by the author to determine the relationships among eight Bovidae and three Cervidae. The value of the serological method in taxonomic studies is controversial. Zuckerman & Sudermann (1935) believe "the serum precipitin test is of limited value in tracing phylogenetic relationship." Boyden, on the other hand, in his publications states that he considers the method an important and reliable tool for phylogenetic studies.

The data presented here offered evidence in support of the idea that the precipitin test can, at times, be of use in the corroboration of morphological facts. Secondly, it seems that it may be a better method for determination of the degree of interrelationships among animals in the smaller taxonomic group, such as an order or family. Especially is this true when certain factors, such as protein concentration of the antigen solutions, are determined and the specificity of the antisera is controlled by injection methods and *in vitro* factors.

Of the eight species of Bovidae whose bloods were tested it was shown that the sheep, goat and tahr were very closely related. Their classification into the subfamily Caprinae would be justified by the serological reactions. The bloods of three antelopes, the eland, black buck and sable (each of which is placed in a separate subfamily) did not give similar reactions with sheep, goat or tahr antisera, the sable antelope reactions being definitely higher, indicating its closer kinship to the sheep, goat and tahr. The degree of this relationship was less than that of the sheep, goat and tahr to each other.

The reactions of the anti-eland sera made possible a distinction of the eland blood from that of all other Bovidae and of the Cervidae. Its relationship values were by far the highest with the ox and buffalo bloods, indicating a closer affinity of the eland to these two animals than to the other animals studied. Beddard states (1920, p. 308), "Such an antelope, however, as the eland, is very ox-like in habit." This similarity is, of course, based on a very superficial characteristic, and the addition of the similar serologic qualities of the animals is important. There was some indication that the eland may be more closely related serologically to the three Cervidae than to the Bovidae, with the exception of the ox and buffalo. This would be contrary to their present taxonomic position.

Several antisera were produced against ox and buffalo blood sera. The results were conflicting, however, and it was believed advisable to postpone a report of the data until further research could ascertain the cause of the discrepancies. The ox and buffalo antisera reactions always showed the very close affinity of the ox to the buffalo, and the value of the cross reaction was almost always 50 to 100 per cent. On the other hand, the titers with the eland, other Bovidae, and the Cervidae were dissimilar and inconsistent. In most instances the eland and Cervidae showed a closer relationship to the ox and buffalo than did the other Bovidae; less often the opposite results were secured.

The antisera produced against the black buck antelope resulted in reactions that easily distinguished it from the other bloods tested. The degree of the heterologous tests suggests that this animal was sufficiently different from the other Bovidae tested to warrant its being classified as a definitive group within the family Bovidae.

The results of the reactions of the anti-Cervidae sera were elaborated upon in the presentation of the data. It was conclusively shown that the American elk and axis deer, an old world deer, were very closely related to each other. The degree of this relationship was of the same order that was found among sheep, goat and tahr, or between ox and buffalo.

In conclusion a brief serologic classification, based upon the values of the Bovidae and Cervidae antisera reactions, is presented. The homologous

serum is considered to belong to Group I and it may be subdivided into subgroups A and B. This group includes, in addition to the homologous blood, those heterologous bloods that consistently gave the highest cross values. The bloods which were usually indistinguishable with the less specific antisera would belong in subgroup A (refer to cross reactions of serum 41 with sheep, goat and tahr). Group II would contain the species whose reactions are consistently lower than those of Group I, and Group III would include the lowest titered reactions. Table 9 presents the provisional serologic classification of the eight Bovidae and three Cervidae.

TABLE 9.

A provisional serologic classification of eight Bovidae and three Cervidae.

Classification based on antisera against sheep, goat and tahr bloods:

Group I
Subgroup A. Sheep, goat, tahr.
Subgroup B. Sable antelope.
Group II—Other Bovidae tested.
Group III—Cervidae.

Classification based on anti-eland sera:

Group I
Subgroup A—Eland.
Subgroup B—Ox and buffalo.
Group II—Other Bovidae tested and Cervidae.

Classification based on anti-black buck antelope sera:

Group I—Black buck antelope.
Group II—Sheep, goat, tahr, sable antelope.
Group III—Other Bovidae tested and Cervidae.

Classification based on anti-ox and anti-buffalo sera:

Group I—Ox and buffalo.
Others doubtful.

Classification based on anti-deer sera:

Group I
Subgroup A—Axis deer and elk.
Subgroup B—Virginia deer.
Group II—Ox and buffalo.
Group III—Other Bovidae tested.

SUMMARY.

1. Nineteen antisera against five species of Bovidae and three species of Cervidae were produced in rabbits.

2. The antigens of fifteen species of Artiodactyla and Perissodactyla were used as test antigens.

3. Many of the antisera which were produced by injections of minute quantities of antigen resulted in very specific antisera.

4. The advantage of the serologic method is that a quantitative relationship could be determined for closely related bloods.

5. The serologic relationships usually agreed with the accepted morphological classification.

6. There were indications that the ox and buffalo were more closely related to the Cervidae than were the other Bovidae tested.

7. The tahr and sable antelope gave higher relationship values with the goat and sheep antisera than did the other Bovidae and Cervidae.

8. The ox and buffalo showed the closest affinity to the eland.

9. The axis deer and elk sera were found to be indistinguishable from each other but distinguishable from the Virginia deer.

Bibliography.

Beddard, Frank Evers.

1920. Mammalia. Cambridge Natural History. Macmillan & Co., Ltd., London.

Boyden, Alan A.

1926. The precipitin reaction in the study of animal relationships. *Biol. Bull.*, Vol. 50, No. 2, p. 73.

Ehrhardt, Albert.

1929. Die Verwandtschaftbestimmungen mittels der Immunitätsreaktionen in der Zoologie und ihr Wert fur phylogenetische Untersuchungen. *Ergebn. u. Fortschr. der Zool.*, Vol. 7, p. 279.

Myers, W.

1900. On immunity against proteids. *Lancet*, Vol. 2, p. 98.

Nuttall, G. H. F.

1904. Blood immunity and blood relationships. Cambridge Univ. Press, Cambridge, England.

Nuttall, G. H. F., & Dinkelspiel, E. M.

1901. Experiments upon the new specific test for blood. Preliminary Note. *Brit. Med. Jour.*, Vol. 1, p. 1141.

Scott, Wm. B.

1937. A history of land mammals in the western hemisphere. Rev. ed. Macmillan Co., New York.

Uhlenhuth, P.

1901. Eine Methode zur Unterscheidung der verschiedenen Blutarten, in besondern zum differential-diagnostischen Nachweise des Menschenblutes. *Deutsche Med. Wochenschr.*, Vol. 27, p. 82.

Wolfe, Harold R.

1933. Factors which may modify precipitin tests in their application to zoology and medicine. *Physiol. Zool.* Vol. 6, p. 55.

1935. The effect of injection methods on species specificity of serum precipitins. *Jour. of Immun.*, Vol. 29, p. 1.

1936. The specificity of precipitins for serum. *Ibid*, Vol. 31, p. 103.

1939. Standardization of the precipitin technique and its application to studies of relationships in mammals, birds, and reptiles. *Biol. Bull.*, Vol. 76, No. 1, p. 108.

Zuckerman, S., & Sudermann, Ann E.

1935. Serum relationships within the family Cercopithecidae. *Jour. Exp. Biol.*, Vol. 12, p. 222.